ISSN print: 0258-5936
ISSN online: 1819-4087

Ministry of Higher Education. Cuba
National Institute of Agricultural Sciences
http://ediciones.inca.edu.cu

ISOLATION AND SELECTION OF AUTOCHTONOUS BACTERIA FROM MANABÍ-ECUADOR WITH CELLULOLYTIC ACTIVITY

Aislamiento y selección de bacterias autóctonas de Manabí-Ecuador con actividad celulolítica

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ABSTRACT. The present study aimed to isolate and select bacteria with cellulolytic capacity, having future application as inoculum in the fibrous organic waste composting. Five sampling environments were considered: organic agriculture (AO), conventional agriculture (AQ), forest (BM); sugarcane area (RC) and compost piles (AC). For bacterial isolation the nutrient agar medium was used changing the carbon source by cellulose. The main selection criterion of the bacterial isolates was the growth on above medium and its positive reaction to congo red test, showing clear zones around the colonies. The 93 bacterial isolates obtained were subjected to Gram staining, catalase test, presence of endospores and aerobically growth; having 70 bacteria *Bacillus* spp. like characteristics. Their cellulolytic activities were determined and 30 bacteria produced hydrolysis halo. Eight of these bacterial isolates were selected according to the largest halo production (AO-19, AO-28, AO-29, AQ-2, BM-7, RC-2, RC-6, CR-18) and their growth at different pH (3, 5, 7, 9) and temperatures (50 and 70 °C) were evaluated. The bacteria AO-19 showed higher hydrolysis halo with 12,33 mm and growth stability at different pH and temperature levels, for which its growth dynamic and amylolytic and pectinolytic capacity was determined. According to results the bacteria AO-19 has potential to be used as inoculum in composting.

Key words: *Bacillus*, bacterial inoculum, composting, ecological plasticity

RESUMEN. La presente investigación tuvo como objetivo aislar y seleccionar bacterias con capacidad celulolítica, que tengan aplicación futura como inóculo en el compostaje de residuos orgánicos fibrosos. Se consideraron cinco ambientes de muestreo: agricultura orgánica (AO); agricultura convencional (AQ); bosque (BM); área cañera (RC) y pilas de compost (AC). Para el aislamiento se usó el medio agar nutriente modificando la fuente de carbono por celulosa. El criterio principal de selección de los aislamientos bacterianos fue el crecimiento sobre el medio antes mencionado y su reacción positiva frente a la prueba de rojo congo, observándose zonas claras alrededor de las colonias. Se obtuvo 93 aislamientos bacterianos a los que se les realizó tinción de Gram, prueba de catalasa; se observó presencia de endosporas y crecimiento en aerobiosis; presentando 70 bacterias características similares a *Bacillus* spp. A las mismas se le determinó actividad celulolítica y se encontró que 30 bacterias produjeron halo de hidrólisis. De ellas se seleccionaron los ocho aislamientos bacterianos que produjeron el mayor halo (AO-19; AO-28; AO-29; AQ-2; BM-7; RC-2; RC-6; RC-18) y se les evaluó su crecimiento a diferentes pH (3, 5, 7, 9) y temperaturas (50 y 70 ºC). La bacteria AO-19 mostró mayor halo de hidrólisis con 12,33 mm y estabilidad de crecimiento en los diferentes niveles de pH y temperatura estudiados; por lo cual se determinó su dinámica de crecimiento y capacidad amilolítica y pectinolítica. De acuerdo a los resultados la bacteria AO-19 posee potencial para ser usada como inóculo en la elaboración de compost.

Palabras clave: *Bacillus*, inóculo bacteriano, compostaje, plasticidad ecológica

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INTRODUCTION

The final destination of organic residues generated worldwide poses a problem that affects the environment. There are current ongoing efforts to reduce the mass of such residues of agricultural, agroindustrial, livestock and municipal rubbish dumps origin by implementing biotechnological methods that contribute to reduce their volume and favor their re-utilization as manure to improve the physical, chemical and biological features of the soils agriculturally suitable (1, 2).

Among the management options is composting, a bio-oxidative process that demands a biological conditioning agent to function, and therefore, it will be affected by very different factors influencing in a higher or lower degree on the optimization of microbial activity. The process implies heterogeneous organic substrates in its composition and homogeneous in its size. During the transformation different stages occur, which finish in reactions of different meaning with the production of intermediate metabolytes that can be phytotoxic; hence the importance of controling maturation. Finally, the composting process leads to the release of CO2, water, minerals and organic matter (OM) more or less stabilized, rich in useful microbial populations and plant physiology bioactivators (3, 4, 5).

In soils, biological diversity functions like a recycler of organic matter, both in the dynamics of nutrient circ ulation as in the energy flows, (6, 7). However, not all microorganisms present can produce the required extracellular enzymes for degradation, mostly fibrous materials which in turn are not affected by physical and chemical factors during hydrolysis. It influences on the required times for humification and mineralization till turning into simpler compounds to be absorbed by plants (3, 4).

The widely accepted mechanism to explain the enzimatic hydrolysis of cellulose and hemicellulose –main components of plant walls- involves (8), the joint action of a set of microbial enzymes out of which the p-1,4 endo-glucanase (endo-(1_4)-B-D-glucanohydrolase), the p-1,4 exo-celobiohydrolase and the p-1,4 glucosidase are the most important ones (9, 10). However, hydrolitic activities are affected by a group of physical and chemical factors that limit the degradation of insoluble cellulose (11, 12); that's why the search for microorganisms with better cellulolytic capacities would make available new biological sources with cellulase activity which could probably be used to treat plant organic residues to produce compost.

Microorganisms that degrade cellulose include fungi, bacteria, aerobial and anaerobial, mesophile and termophile, that cover a series of habitats. Soils are considered the most diverse environments on earth from the microbial point of view due to their great physical, chemical and biological heterogeneity. For instance, up to 109 bacteria can be found in one gram of soil of horizon A, with a diversity of more than 4 600 different genomes, since the abundance, composition and diversity of the microbial communities on the soil are heavily related with depth (13).

Among the cellulolytic bacteria those of the genera *Cellulosomas* sp, *Pseudomonas* sp., *Cytophaga* sp. and *Bacillus* sp. (14, 15) stand out. No doubt that native organisms have advantage over those introduced whose action is more effective since they do not need to adapt to climate, moisture and microorganisms populations already present in the area where they are used (9, 10).

In Ecuador, and particularly in Manabí, imported inoculants are sold as accelerators of the composting process. However, the label of such inoculants does not show the origin, identification and specie of those microorganisms, so it is not possible to clearly show the results of using them (16). This research aimed at isolating, selecting and characterizing native bacteria of this location with cellulotytic activity and potential to produce bioproducts by using them in the production of quality compost.

MATERIALS AND METHODS

Collection of soil samples

This activity was developed in a wide Tropical Subhumid area at the Manabí province, Ecuador. This zone records an average temperature of 28 °C and around 800 mm annual rainfall distributed on a six-month rainy period (December-May).

The sampling environments were: 1) organic agriculture area (OA)-devoted to agroecological practices of horticultural polycrops, constant incorporation of organic matter to the soil, fresh and stabilized and a value of around 4,06 % of OM; 2) conventional agricultural area (CA)-in which short-cycle intensive crops are planted using chemical-mechanized agronomical practices, good drainage and 1,37 % of OM; 3) artificial forest (BM)-made up of mainly wood species: mahogany, "guachapelí" and oak, of approximately 20 years old that has generated a leaf litter of nearly 5 cm over the soil; there is spontaneous vegetation associated, low growing grasses and shadow-tolerant dicotyledoneous plants, the soil has 4,05 % of MO. These three environments are used for students' practice and belong to the campus of the Higher Polytechnical Agricultural School of Manabí "Manuel Félix López" (ESPAM-MFL), located at cantón Bolívar.

The other environments were: 4) sugarcane plot (RC)-of approximately 10 years old. Single-crop exploitation, it has an evident accumulation of fibrous residues, high moisture retention and 1,61 %

of OM in the soil; the location belongs to cantón Chone; 5) compost production unit (AC)-protected environment and cement floor where high volumes of organic residues are composted using machinery to remove and dump raw materials, composting system is open and there are piles in different stages of the process. The one selected for sampling had eight weeks of duration and 25,51 % of OM; this infrastructure belongs to the organic banana unit "Nueva Esperanza" located at cantón Tosagua.

Out of each environment, a sample composed of 500 g was taken from three sampling sites at 10 cm deep, selected by the favorable habitat for the ecological niche of cellulolytic microorganisms (15, 17).

Preliminary isolation and selection of bacteria for the different environments

Samples were processed at the Molecular Biology Lab of ESPAM "MFL". For isolation, 1 gram of substrate from samples taken in each of the environments was used, and the methodology of serial dilutions was followed (18). A modified nutrient agar medium was also used (19) containing peptone 0,6 %, yeast extract 0,2 %, sodium chloride 0,5 %, amorph cellulose (Himedia) 1,5 % and agar 1,5 %. pH was adjusted at 7,3. Dilutions were done (1:10) till 10-6 and those from 10-4 till10-6 were sown three times. Later on, they were incubated for 24 hours at 37±2 °C. All colonies with different morphological features were selected and sown on pipes with nutrient agar for their conservation and further use.

All isolated bacteria were submitted to Gram dying, catalase test, aerobiosis growth (20) and endospores dying (21). Those resulted in Gram positive bacillus, with endospore presence, positive catalase and that grew under aerobiosis conditions, were selected.

Qualitative determination of the cellulolytic capacity

The selected bacterial isolates were inoculated on dishes with nutrient agar supplemented with Carboximethylcellulose (CMC), 1% (p/v). Such medium was composed of CMC 1,0 %, yeast extract 0,25 %, peptone 0,25 %, ammonium sulphate 0,05 %, calcium chloride 0,05 %, monobasic potassium phosphate 0,01 %, dibasic potassium phosphate 0,01 % and agar 1,5 %; the pH of the medium was adjusted at 7,0 and was incubated at 37 °C for 72 hours. Upon incubation, the CMC degradation was developed by covering the medium with congo red solution 1,0 % (p/v). The coloring agent stopped working for 15 minutes, the excess was removed and it was washed twice with sodium chloride 2 mol L^{-1}, providing it with 15 minutes rest. After this time,the cellulolytic activity was determined by the presence of halo areas expressed by the hydrolysis of the cellulose whose diameter was measured in mm (22, 23).

Growing capacity of the degradating bacterial isolates of cMc

When evaluating growth capacity, bacterial isolates were freshened up inoculating an bunch of each sample in 25 mL of nutrient medium: this medium was composed of peptone 1.0%, yeast extract 1,0 %, sodium chloride 0,5 %, pH adjusted to 7,3. It was incubated for 24 hours at a temperature of 37 °C; 5 mL were taken from this suspension and were added to 50 mL of nutrient medium from where a sample to evaluate at the spectrophotometer was taken (Jenway 6305), the optical density used was at a wave length of 660 nm, 24 hours later.

Growth capacity at different pH and temperature values of the bacterial isolates with cellulolytic capacity

Bacteria producing the largest halo of cellulose hydrolysis were selected as the primary criterion of selection and the growth dynamics was evaluated at different pH and tempetatures.

pH

Selected bacteria were cultured on a nutrient medium with pH 7,3, they were incubated at 37 °C for 18 hours and agitated at 130 rpm (24). Out of each culture, 5 mL with 1010 UFC.mL^{-1} was taken and inoculated in erlenmeyers of 125 mL of effective capacity and 45 mL of nutrient medium. The pH of the medium in erlenmeyers was previously adjusted at 3-5-7-9 with HCl 0,1 N (25).

All samples were incubated at 37 °C for 24 hours. Simultaneously, the control was implemented in the nutrient medium at a pH of 7,3 under the same conditions of the treatments. Three replicates per culture were done.

At the inoculation time, at cero and 24 hours, viable cells were counted through serial dilutions on a sterile saline solution, dilutions were sown three times from 10-9 and 10-11 in dishes with nutrient agar. Incubation was done at 37 °C for 24 hours. Survival percentage was calculated b y the equation of Kociubinski (26).

$$\%\ R\ pH = [(UFC\ mL^{-1}\ a\ las\ 24\ h)\ CN\ pH\ 3 \times 100] / (UFC\ mL^{-1}\ at\ cero\ hour)\ cn\ pH\ 7,3$$

The same formula was repeated for each of the studied pH. A bacterial growth percentage higher or equal to 50% at 24 hours of incubation was taken as the selection criterion.

Temperature

Bacterial isolates were cultured in nutrient medium with pH 7,3 and incubated at 37 °C for 18 hours, under agitation at 130 rpm (23). Our of each culture 5 mL with 1010 UFC mL^{-1} were taken and incubated in erlenmeyer of 125 mL of effective capacity with 45 mL

of nutrient medium with pH 7,3 and were incubated at 50 and 70 °C for 24 hours. Later on, viable cells were counted by the technique of serial dilutions (18) on a sterile saline solution. Then dilutions from 10-9 to 10-11 were sown three times on dishes with nutrient agar.

Characterization of the selected bacteria

The bacterial isolate that showed the best values according to the above-mentioned parameters, was characterized as follows:

Growth

A growth dynamics was done at 0, 4, 8, 12, 16, 20 and 24 hours, from fresh culture in nutrient agar wedges inoculated with nutrient medium (1:10) in erlenmeyers of 100 mL capacity containing 50 mL of medium. They were incubated on a thermostated sieve at 37 °C and 130 rpm of agitation (24). At the indicated times, erlenmeyers were removed to count viable cells by the serial dilution technique on a sterile saline dilution. Dilutions from 10-9 yo 10-11 were sown three times in dishes with nutrient agar. Incubation was done at 37 °C for 24 hours.

Amilolytic and pectinolytic activity

In order to determine the amilolytic activity, the selected bacteria was inoculated in dishes with nutrient-starch agar at 1,0 % (p/v) (27). This culture medium was composed of soluble starch 1,0 %, yeast extract 0,25 %, peptone 0,25 %, ammonium sulphate 0,05 %, calcium chloride 0,05 %, monobasic potassium phosphate 0,01 %, fosfato dibasic potassium phosphate 0,01 % and agar 1,5 %. The pH was adjusted to 7,0 and incubation was at 37 °C for 72 hours. Degradation was developed using a lugol solution at 0,50 % (p/v), during two minutes till covering the medium for a better observation of the digestion halo. As negative control, a dish of nutrient agar with starch at 0,2 % (p/v) without inoculation was used, and as positive degradation control an isolate of *Bacillus cereus* ATCC-14579 (28, 29) was used.

In order to determine the pectinolytic activity, the selected isolate was inoculated in pectin agar at % (p/v) (27). This culture medium was modified with a composition based on soluble pectin at 1,0 %, ammonium sulphate 0,05 %, calcium chloride 0,05 %, monobasic potassium phosphate 0,01 %, dibasic potassium phosphate 0,01 % and agar 1,50 %. The pH was adjusted to 7,0 and incubation was done at 37 °C for 72 hours. The pectinolytic activity was determined by the presence of light areas around the colonies due to pectine hydrolysis and development with lugol. As positive control of the degradation, an isolate of *Bacillus cereus* ATCC-14579 was used (28, 29).

Statistical analysis

For data processing the software INFOSTAT version 1 (30) was used. Analysis of variance were done to determine significant differences of the variants. Tukey's test was used to make multiple comparisons among means. The counting of viable microorganisms was transformed to a Log N, to guarantee normal conditions in the growth curve. The analysis took into account the formula (K+N)*10x, where K is the constant representing dilution logarithm in which microorganisms were inoculated; N is the logarithm of the UFC number and x is the dilution inoculation was done to. When counting was equal to cero a constant was added (x + 0,375).

RESULTS AND DISCUSSION

Preliminary isolation and selection of bacteria from different environments

A total of 93 bacteria (Table I) were isolated. As to sampling sites, the sugarcane plot (RC) resulted a good habitat for bacteria of interest, probably because the fibrous residue deposit favors the presence of microorganisms with cellulolytic capacity. In this regard, it is essential to consider the type of sample and sampling zones as an important conditions to isolate bacteria with high degradating possibilities of cellulolytic residues (31).

Table I. Bacteria isolated under different environments

Environments	Number of bacterium	%
Organic agriculture	26	28,0
Conventional agriculture	7	7,5
Forest	12	13,0
Sugarcane plot	41	44,0
Composting	7	7,5
Total	93	100

The second best percentage of bacterial isolates stemmed from the organic area (OA), which could be favored by the incorporation of organic manure and conservation practices as part of the ecological soil management (6, 7). A similar performance was true for the forest environment (F), where a considerable number of bacterial isolates was attained. This result is related to the high number of bacteria reported in studies of population dymamics carried out in soils of tropical forests with a high content of fibrous organic matter with different decomposition degrees (15, 16, 32).

The convencional area (CA) showed the lowest number of bacterial isolates, being the chemical and mechanized practices done to the soil a `possible influence for such a low level of organic matter and therefore, the population dynamics of decomposing microorganisms (33, 34).

Likewise, compost (AC) resulted in a similar quantity of bacterial isolates compared to the conventional environment despite the high content of organic matter of the compost. This could explain that seemingly the compost sample was taken from a pile in an advanced stage of stabilization and maturity of composting where the degrading activity of cellulose and microorganisms is reduced (4, 35).

Ot of the 93 bacterial isolates, 70 were Gram-positive bacillus, positive catalase, endospore-forming and grew up in aerobiosis. These features are related to bactaria belonging to the *Bacillus genus*; according to the classic method of identifiying microorganisms that uses phenotipical, morphological and physiological characters as differentiation criteria (21, 36). The observation of morphology and sporulation, the response to Gram dyeing and some biochemical tests allow, in the case of *Bacillus* spp., placing them into the genus (37).

Qualitative determination of the cellulolytic capacity and microbial growth

Table II shows the 30 bacterial isolates que positively responded to cellulolytic. Fifty seven percent of these bacteria belong to the sucarcane area; from the organic and conventional area 13 % was attained; the rest 17 % corresponds to the forest environment. Bacterial isolates from compost did not show cellulolytic activity which is in accordance to other studies where a low number of bacteria with cellulolytic activity was attained by using agar CMC for isolation from advanced composting of crops like Stevia rebaudiana and different natural substrates from pasture lands and tropical flooded sabana forests (22, 38).

In general, bacterial isolates showed a differential behavior for the two evaluated variables. The bacterium AO-19 showed the highest degrading halo of cellulose with 12,33 mm, being statistically different

Table II. Cellulolytic activity per hydrolysis halo and microbial growth

Environments	Microbial isolate	Hydrolysis Halo (mm)	SD ±	Growth (24 hours) DO (660 nm)	SD ±
Organic area	AO-19	12,33 a	0,76	9,63 abc	0,15
	AO-28	3,42 bc	2,98	9,42 abcde	0,13
	AO-29	3,42 bc	2,25	9,23 bcdef	0,04
	AO-30	2,83 c	2,36	9,13 cdef	0,12
Conventional area	AQ-2	3,23 bc	1,08	9,71 abc	0,05
	AQ-3	1,33 c	0,58	9,90 a	0,07
	AQ-4	1,67 c	1,53	9,16 cdef	0,11
	AQ-8	2,37 c	0,51	9,58 abcd	0,03
Forest	BM-1	1,17 c	1,26	9,91 a	0,03
	BM-5	2,50 c	1,37	9,97 a	0,02
	BM-7	3,50 bc	1,09	8,79 fgh	0,17
	BM-10	2,33 c	2,10	9,52 abcd	0,14
	BM-11	1,50 c	0,87	9,42 abcde	0,13
Sugarcane plot	RC-1	2,33 c	0,29	8,97 defg	0,08
	RC-2	4,73 bc	2,11	9,90 a	0,03
	RC-3	2,33 c	0,29	9,57 abcd	0,05
	RC-4	1,83 c	1,61	9,94 a	0,05
	RC-5	1,33 c	1,15	8,68 fghi	0,15
	RC-6	7,50 b	2,00	9,98 a	0,01
	RC-7	2,83 c	0,29	9,15 cdef	0,11
	RC-8	2,17 c	1,26	8,15 i	0,04
	RC-9	2,33 c	0,29	8,17 i	0,07
	RC-10	2,17 c	0,29	8,41 ghi	0,08
	RC-11	2,00 c	1,00	9,81 ab	0,24
	RC-12	2,17 c	1,89	8,24 hi	0,16
	RC-13	2,33 c	0,29	9,90 a	0,02
	RC-15	2,33 c	0,29	8,35 efg	0,89
	RC-16	2,67 c	0,29	8,48 ghi	0,06
	RC-18	3,50 bc	0,87	9,60 abc	0,11
	RC-23	1,17 c	1,26	9,91 abc	0,07
Positive control	*Bacillus cereus* ATCC-14579	4,92	1,94	8,80	0,15
Probability		<0,0001		<0,0001	
Standard Error		0,79		0,11	

SD: standard deviation

from the rest of bacterial isolates in addition to exhibit a high growth on the medium though without differences regarding some of the studied bacteria.

Bacterial isolates found in the first two statistical categories in the hydrolysis halo variable (AO-19; AO-28; AO-29; AQ-2; BM-7; RC-2; RC-6; RC-18) were selected to evaluate growth capacity at different pH and temperature levels, since they are two very important environmental factors that come up with different values during the composting stages of organic residues (3, 4). The other 22 isolates share the third statistical category by having hydrolysis halo averages below 3 mm. These results of cellulose degrading coincide with those from other studies that did not surpass the above-mentioned value so they were rejected as possible inoculum (22, 39).

Effect of the pH of the culture medium over the growth capacity of selected bacterial isolates

Selected bacterial isolate growth is shown in Table III. It can be seen that isolate AO-19 exhibited the highest growth for different pH values of the culture medium reaching an average resistance percentage of 77,5 %. It turns this bacteria into the one with the highest ecological plasticity; it is also confirmed that neutral and alkaline pH in the culture medium are the optimum ones for bacterial growth of the genus *Bacillus* (14, 15, 25). It should ne noted that all bacteria grew up on acid pH coinciding with other studies done to 43 *Bacillus* spp., which reported that 100 % of the evaluated isolates grew up at a pH of 4, 5 and 6 (33).

Incubation temperature effect on the growth of selected bacterial isolates

Table IV shows the results of bacterial growth; the isolate AO-19 was among those withg the highest growth under the two temperatures studied. This is a very important condition to consider for microbial inoculum because composting involves high temperatures that are indicators of the microbial activity in the transformation of organic matter, mostly in the termophile stage where fibrous compounds decomposition occurs. (9, 10, 17).

Table III. Resistance percentage of the bacterial isolates at different pH values

Bacterial isolates	pH 3		pH 5		pH 7		pH 9	
	% R	SD	% R	DS	% R	SD	% R	SD
AO-19	60,0 a	0,82	68,0 a	1,63	92,7 a	2,05	90,0 a	0,82
AO-28	32,0 cde	1,63	43,7 c	1,24	62,0 b	2,16	59,3 b	1,25
AO-29	25,0 e	3,26	52,7 b	1,70	53,0 c	2,16	49,3 d	2,49
AQ-2	32,3 cd	2,05	40,0 cd	1,63	57,7 bc	1,25	53,3 dc	1,24
BM-7	31,0 de	1,63	33,0 de	2,45	63,0 b	2,45	57,0 bc	1,63
RC- 2	39,0 bc	1,63	53,0 b	2,45	88,0 a	1,63	85,0 a	1,63
RC- 6	46,0 b	2,45	56,0 b	3,68	90,0 a	0,81	88,0 a	1,63
RC-18	29,0 de	2,45	30,0 e	1,63	59,0 bc	2,45	55,0 bc	1,63
Probability	<0,001		<0,001		<0,0001		<0,001	
SE	1,49		1,54		1,38		1,14	

R: resistance SE: standard error SD: Standard deviation

Table IV. Bacterial isolates growth at different temperatures

Bacterial isolates	50 °C		70 °C	
	Ln UFC mL^{1}	SD	Ln UFC mL^{1}	SD
AO-19	22,9 a	1,63	21,7 a	0,72
AO-28	19,5 ab	0,73	17,2 abc	1,33
AO-29	21,5 ab	0,69	19,2 ab	0,59
AQ-2	13,7 bc	0,83	17,1 d	1,40
BM -7	18,8 ab	1,59	15,6 bcd	1,76
RC-2	18,7 ab	1,32	18,8 ab	1,60
RC-6	16,5 abc	4,90	11,8 cd	3,27
RC-18	8,7 c	1,79	3,0 e	1,18
Probability	<0,0011		<0,001	
EE	1,79		1,18	

SE : Standard error SD: Standard deviation

Characterization of the bacteria AO-19

Results indicate that bacteria AO-19 is the most promising one as per the aspects evaluated, mainly to the main criterion selected that is the degradation of the cellulose (Figure 1), so it was characterized as to:

Growth dynamics of the bacterial isolate AO-19

Figure 2 shows the growth curve of the promising bacteria AO-19. In the early hours, the growth of the culture starts and maintains in the logarithm stage till 18 hours. Till 24 hours, cells stopped multiplying because of reduced nutrients and increased toxic substances in the medium (36, 40).

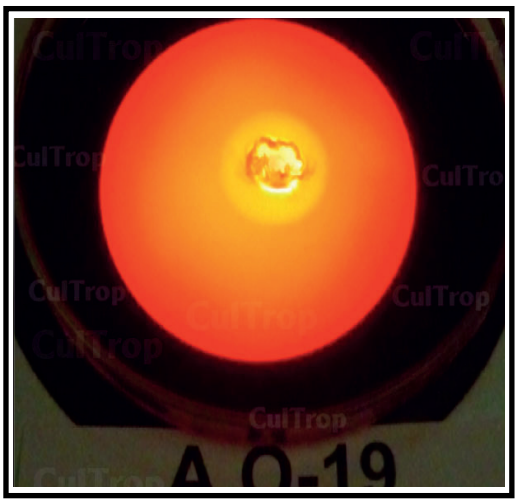
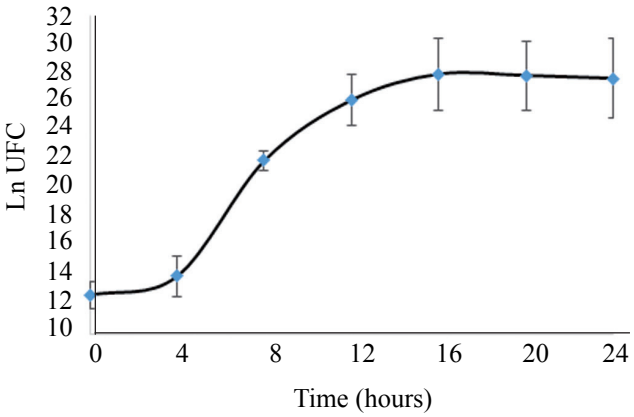


Figure 1. Image of the hydrolysis halo of the carboximethylcellulose (CMC) by the bacteria AO-19 en nutrient agar, expressed like a light area around the bacteria



Bars indicate the standard deviation

Figure 2. Growth curve of the bacteria AO-19 at 37 °C in nutrient medium

Amilolytic and pectinolytic activity of the bacterial isolate Ao-19

Table V shows the values of hydrolysis halo indicating amilolytic and pectinolytic activity of the AO-19 bacteria, reaching 8,08 mm and 1,7 mm, respectively; it falls into the interval from 1,5 to 15,0 mm reported in other studies (5, 41). The AO-19 isolate surpasses in amilolytic capacity the positive control and reached an halo diameter very similar to pectin's. These qualities are very important at the time of deciding on the types of bacteria to inoculate in composting processes based on the chemical composition variable of organic residues (14, 15).

Table V. Amilolytic and pectinolytic capacity of the bacterial isolate AO-19

Bacteria	Starch hydrolysis halo		Pectin hydrolysis halo	
	mm	±SE	mm	±SE
AO-19	8,08	1,09	1,7	9,93
Positive control *Bacillus cereus* ATCC-14579	6,41	1,25	1,83	0,47

CONCLUSIONS

Under the climatic conditions of Manabí- Ecuador, soils devoted to sugarcane production have a high microbial load of cellulolytic bacteria. However, out of the soil where organic agriculture is practiced, the bacterial isolate AO-19 was obtained with a higher degrading capacity of cellulose and better growth at different pH levels and temperature, which are desirable and necessary features for the inoculum of composting so it is recommended for such purpose.

ACKNOWLEDGEMENT

To the Higher Polytechnical Agricultural School of Manabí "Manuel Félix López" for sponsoring and financing this research. The Lay University "Eloy Alfaro" from Manabí-Ecuador, which provided the facilities for this study. Likewise, our gratitude to the experts of the Faculty of Agronomy of Matanzas' University "Camilo Cienfuegos" in Cuba for their scientific contribution to this research.

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Received: July 19th, 2013
Accepted: April 15th, 2014

Revista Cultivos Tropicales
Instituto Nacional de Ciencias Agrícolas